HHS Public Access

Author manuscript

*Histopathology*. Author manuscript; available in PMC 2025 January 01.

Published in final edited form as:

*Histopathology*. 2024 January ; 84(2): 387–398. doi:10.1111/his.15074.

# Expanding the histologic spectrum of salivary gland neoplasms with *HMGA2::WIF1* fusion emphasizing on their malignant potential: a report of eight cases

**Nora Katabi, MD**[1], **Purvil Sukhadia, MD**[1], **Sara E. DiNapoli, PhD**[1], **Ilan Weinreb, MD**[2], **Elan Hahn, MD**[3,4], **Ronald Ghossein, MD**[1], **Bin Xu, MD PhD**[1]

[1]Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[2]Department of Pathology, University Health Network, Toronto, ON, Canada

[3]Department of Pathology and Laboratory Medicine, Sinai Health System, Toronto, ON, Canada

[4]Department of Laboratory Medicine and Pathobiology, University of Toronto, ON, Canada

## Abstract

**Aims:** Recently, *HMGA2::WIF1* fusion have been reported in pleomorphic adenoma (PAs) originating from the parotid gland with a characteristic canalicular adenoma-like pattern. However, it is unclear whether *HMGA2::WIF1* fusion may occur in salivary gland carcinoma or tumors originating from the minor salivary glands. We herein conducted a detailed clinicopathologic review of eight salivary gland tumors harboring *HMGA2::WIF1* fusions.

**Methods and results:** The reviewed diagnoses of salivary gland neoplasms with *HMGA2::WIF1* fusion were PA (n=4), myoepithelioma (n=1), myoepithelial carcinoma ex PA (n=2), and high-grade carcinoma with basaloid features (n=1). Two tumors originated from the minor salivary glands. Six tumors (80%) contained areas reminiscent of canalicular adenoma characterized by interconnected trabeculae/canaliculi of monotonous oncocytic or cuboidal tumor cells associated with a hypocellular, hyalinized to myxoid stroma. Areas typical of PA were seen in 4 (50%) cases. All tumors showed diffuse S100 and CK7 immunopositivity. Adverse events were detected in two cases, including local recurrence in a patient with PA, and local and distant

**Correspondence:** Bin Xu, MD, PhD, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, xub@mskcc.org.

Authors' contributions:
Study design: BX
Case contribution and pathology review: NK, BX, RG, IW
Clinical review: BX
Molecular analysis and detection of HMGA2::WIF1 fusion: PS, SED, EH
Manuscript drafting: BX
Manuscript editing: NK, PS, SED, IW, EH, RG, BX

**Conflict of interest:** No competing financial interests exist for all contributory authors.

**Ethics approval statement:** The research is approved by the Institutional Review Board (IRB) of each participating site.

**Patient Consent statement:** Not applicable.

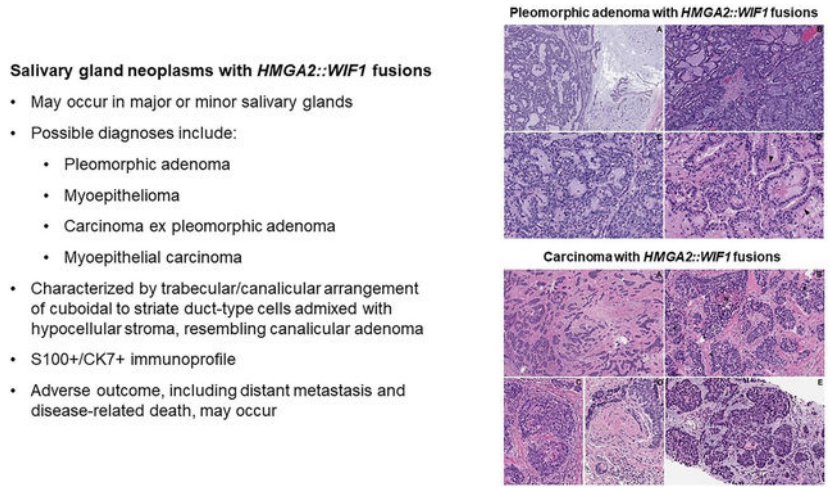
**Permission to reproduce material from other sources:** Not applicable.

**Clinical trial registration:** Not applicable.

recurrences and disease-related death in a patient with a high-grade carcinoma of the minor salivary gland of the buccal space, showing tumor necrosis and perineural invasion.

**Conclusion:** Salivary gland neoplasms with *HMGA2::WIF1* fusion are predominantly characterized by canalicular adenoma/striated duct adenoma-like histology and a S100+/CK7+ immunoprofile. These tumors are not always benign, as among all reported cases, approximately 20% showed malignancy (6/28) and adverse outcome (3/15), including recurrence, distant metastasis, and disease-specific mortality.

## Graphical Abstarct




Salivary gland neoplasms with *HMGA2::WIF1* fusions may be malignant or benign, characterized by canalicular adenoma/striated duct adenoma-like histopathology and a S100+/CK7+ immunotype. Diagnoses include pleomorphic adenoma, myoepithelioma, myoepithelial carcinoma, and carcinoma ex pleomorphic adenoma.

### Keywords

Salivary gland neoplasm; *HMGA2::WIF1* fusion; pleomorphic adenoma; myoepithelial carcinoma

## Introduction

Among salivary gland neoplasms, pleomorphic adenoma (PA) and carcinoma ex pleomorphic adenoma (CA ex PA) are characterized by diagnostic translocations involving *PLAG1* or *HMGA2* genes [1, 2]. In 2007, *HMGA2::WIF1* fusion was first described in a PA and a CA ex PA by Queimado et al. [3]. Afterwards, *HMGA2::WIF1* fusion was reported in two additional malignant cases: the first was reported by Persson et al. as a malignant transformation of a recurrent PA to CA ex PA [4]; and the second was reported by Ihrler et al. as an adenoid cystic carcinoma with sarcomatoid transformation ex PA [5]. However, the histologic features and immunoprofile of the three documented malignant cases were not provided. To date, *HMGA2::WIF1* fusion has been reported in 20 cases of salivary gland neoplasms in the English literature [3–7], most of which had a diagnosis of PA (17/20, 85%).

Recently, Agaimy et al. described the histologic features of 12 PAs harboring *HMGA2::WIF1* fusion, all of which originated from the parotid gland and exhibited characteristic histology with interconnecting trabeculae/canaliculi lined by monomorphic bilayered or multilayered tumor cells, resembling canalicular adenoma (CAA) or trabecular myoepithelioma. This characteristic histologic pattern was described by the authors as a "canalicular adenoma-like" morphology. In this series, areas typical of PA were seen in 50% (6/12) of cases. No adverse events (e.g., recurrence, metastasis, or mortality) were reported, but follow-up data was only available for a subset of cases (n=5, 42%) with a relatively short follow-up period (median=5 months, up to 18 months).

In the current study, we reported detailed clinicopathologic features, immunoprofile, and outcomes of eight salivary gland neoplasms harboring *HMGA2::WIF1* fusion, including five benign tumors and three carcinomas. Additionally, a literature review of salivary gland neoplasms with *HMGA2::WIF1* fusion was conducted, aiming to highlight the possibility of malignant diagnoses and adverse outcomes in these tumors.

## Materials and Methods

### Case selection and clinicopathologic review

The study was approved by the Institutional Review Board of each participating site. The RNA sequencing archive and pathology database of Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY, USA) and Mount Sinai Hospital/University Health Network (MSH/UHN, Toronto, ON, Canada) were searched for salivary gland neoplasms harboring *HMGA2::WIF1* fusions. A total of eight cases were identified (MSKCC: n=7, MSH/UHN: n=1). A consensus diagnosis was provided for all cases at a consensus conference iamong NK, RG, and BX. Detailed pathologic parameters, as well as clinical information, including treatment and outcome data, were collected.

Immunohistochemical studies were performed using the following antibodies: S100 (polyclonal, Leica, ready to use RTU), calponin (clone: EP798Y, Cell Marque, RTU), CK7 (clone: OV-TL-12/30, DAKO, dilution 1:800), CK5/6 (Clone XM26, BioCare, RTU), p63 (clone 4A4, BioCare, RTU), p40 (clone BC28, BioCare, dilution 1:400), and Ki67 (Clone MIB-1, Dako, dilution 1:200).

### Detection of *HMGA2::WIF1* fusion

*HMGA2::WIF1* fusion was detected as part of routine clinical diagnostic work up to classify salivary gland tumors, using either the ARCHER RNA sequencing platform (n=7) or the Illumina TruSight RNA fusion panel (n=1). The ARCHER platform is a clinical molecular diagnostic assay performed in a CLIA-accredited laboratory that utilizes multiplex polymerase chain reaction (PCR) to detect oncogenic fusion transcripts involving 123 genes, as described previously [8]. The Illumina TruSight RNA platform has been previously described [9, 10]. In brief, RNA-seq libraries were prepared using the Illumina TruSight RNA fusion panel, which targets 507 known fusion-related gene targets (Illumina, San Diego, CA, US). The tumor sample was sequenced using the Illumina MiSEq V.3

platform, and fusion gene analysis was performed using the STAR and BOWTIE2 aligners, as well as the Manta and JAFFA fusion analysis tools, respectively.

## Results

The clinicopathologic features of the salivary gland neoplasms with *HMGA2::WIF1* fusion are summarized in Table 1. There was a female predominance with a female-to- male ratio of 1.7:1 (5:3). The median age of presentation was 65 years (range: 36 – 83). Although these tumors tended to occur in major salivary glands, such as the parotid gland (n=4) and the submandibular gland (n=1), minor salivary glands of parapharyngeal space and buccal region (one each) might also be affected. Among the seven cases undergoing surgical resection, the median tumor size was 2.4 cm (range: 1.2 – 3.7 cm).

The consensus diagnosis for these tumors was benign in five cases and malignant in the remaining three cases. The benign diagnoses included PA (n=4) and myoepithelioma (n=1). The malignant tumors were myoepithelial carcinoma ex pleomorphic adenoma (n=2) and high-grade carcinoma with basaloid features (n=1). All patients were devoid of lymph node metastasis clinically and/or pathologically at the time of initial resection.

Seven cases underwent resection, while the remaining one was a biopsy. Among the resected tumors, six were submitted entirely for histologic examination, whereas one (Table 1, Case 2) was sampled representatively at a rate of four tumor sections per centimeter of tumor. Four (50%) of them contained areas with typical histologic features of PA, characterized by myxoid to chondromyxoid stroma with dispersed ductal and myoepithelial elements (Figure 1A). The most common histologic pattern observed in salivary gland tumors with *HMGA2::WIF1* fusion was canalicular adenoma-like, and it was the dominant histologic feature in six cases (75%). These CAA-like areas were characterized by monotonous cellular proliferation arranged as interconnected trabeculae or canaliculi (Figure 1B). Distinct lumen could be seen in the canaliculi, distinguishing them from solid trabeculae. The canaliculi were lined by a monolayered, bilayered, or multilayered epithelium. The lining cells were oncocytic with abundant eosinophilic cytoplasm, centrally located nuclei, small distinct nuclei, and a suggestion of basal striation, resembling native striated ducts; or cuboidal cells with or without spindling with scanty cytoplasm (Figures 1C and 1D). The stroma in-between the canaliculi/trabeculae was consistently hypocellular, presenting as edematous, hyalinized, or myxoid (8/8, 100%).

Only a single tumor (myoepithelial-rich PA, case #2) exhibited typical histologic features of a PA and entirely lacked a CAA-like histologic pattern (Figures 2A–2C, case #2). In one case, due to its high-grade features, the carcinoma with basaloid features did not display an obvious CAA histology, but areas with a trabecular arrangement and intervening hypocellular hyalinized to myxoid stroma reminiscent of the CAA -like pattern were observed (Figure 3, case #6).

Other histologic features that were occasionally observed in salivary gland tumors with *HMGA2::WIF1* fusions included areas resembling fibroadenoma of the breast, characterized by abundant hypocellular stroma surrounded by compressed lining (n=3, Figure 2D), and

basal cell adenoma-like areas with basaloid trabeculae and prominent peripheral palisading (n=1, Figure 2E).

Immunophenotypically, these tumors, whether malignant or benign, showed diffuse positivity for S100 (8/8 cases, 100%, Figure 1E) and CK7 (3/3 cases, 100%, Figure 1F). The CAA-like areas might exhibit calponin expression, ranging from rare cells to focal expression (Figure 1G), suggesting that the lining cells may possess (modified) myoepithelial features. High molecular weight cytokeratins, such as CK5/6, were positive in all tested cases (4/4, 100%), but their expression could be focal. These tumors displayed a p63-positive (5/5, 100%)/p40-negative (0/5, 0%) pattern, although p63 immunopositivity was rare or focal.

Three cases received a malignant diagnosis. The first case (case #6, table 1) was a high-grade carcinoma originating from the buccal area of a 60-year-old female patient. The tumor exhibited basaloid features, focal squamous differentiation, elevated mitotic count of 15 per 2 $mm^2$ (10 high power fields), focal tumor necrosis, and perineural invasion (Figures 3A–3E). No obvious pre-existing PA was identified histologically. The patient developed local recurrence in 5 months, distant metastasis to lung in 31 months, and succumbed to her disease in 73 months. The other two malignant cases (Table 1, Supplementary Figure 1, case #7, Figures 3F–3H; and case #8, Figures 3I–3J) were diagnosed as malignant (myoepithelial carcinoma ex pleomorphic adenoma), based on either the presence of vascular invasion (case #7) or an invasive multinodular myoepithelial growth with multiple capsular protrusions (case #8). Both tumors contained typical areas of pleomorphic adenoma and nodular proliferation of myoepithelial cells arranged as trabeculae (CAA-like areas).

Clinical outcomes were available for five cases, and adverse events were observed in two of them. Apart from the patient with high-grade carcinoma who developed distant metastasis and ultimately succumbed to the disease, a patient with a prior diagnosis of pleomorphic adenoma experienced local recurrence. Unfortunately, the primary tumor was not available for review. The remaining four patients were alive without any evidence of recurrence at their last follow-up.

Additionally, DNA sequencing using MSK-IMPACT platform was performed for case #6, revealing additional molecular alterations. These included *CDK4* and *MDM2* amplification in the primary tumor, as well as *SUFU* somatic mutation and amplification of *CDK4, MDM2, MYC,* and *AURKA* in the lung metastasis.

## Discussion

In this case series, we provide a detailed description of the clinicopathologic features of eight salivary gland neoplasms harboring *HMGA2::WIF1* fusions, three of which were malignant. To date, including the cases presented in this series, a total of 28 salivary gland tumors with *HMGA2::WIF1* fusions have been reported in the English literature (Summarized in Table 2) [3–7]. While the majority of these tumors are benign (n=22, 79%), the rate of malignancy is not insignificant, being reported in six (21%) patients, including a case of malignant transformation from PA to CA ex PA at the time of second recurrence and

five cases of primary carcinomas. The reported histotypes of these malignant tumors include CA ex PA in five cases and a high-grade carcinoma with basaloid features in one. Within the CA ex PA category, the carcinoma component is identified as myoepithelial carcinoma (n=2), adenoid cystic carcinoma with a sarcomatoid component (n=1), and not described (n=2). Among the 15 reported cases with follow-up data, adverse events have been observed in three (20%), encompassing local recurrence (n=3), regional recurrence (n=1), distant metastasis (n=1), and disease-related mortality (n=2). These findings further underscore the malignant potential of these tumors.

Clinically, salivary gland neoplasms with *HMGA2::WIF1* fusions are most commonly observed in the parotid gland (n=17, 81%). However, submandibular gland (n=1, 5%) and minor salivary glands (n=3, 14%) may also be affected. These tumors tend to occur patients in their 60s, with a median age of 66 years (range: 36 – 87). There is a slight female predominance, with a female-to-male ratio of 1.17:1.

Histological and immunohistochemical features of these tumors have been previously described in 20 cases by Agaimy et al. [7] and are further confirmed in the current study. In this study, we corroborated the findings reported by Agaimy et al. that *HMGA2::WIF1*-translocated salivary gland tumors are predominantly characterized by a dominant CAA-like histologic pattern. Almost all cases (95%) contained CAA -like areas. These CAA-like areas consist of interconnected trabeculae or canaliculi formed by monotonous tumor cells arranged as a monolayer, bilayer, or multilayer. The cytology of the tumor cells varies, ranging from oncocytic with suggestions of basal striation resembling striated duct cells, to cuboidal with scanty cytoplasm, and occasionally displaying slightly spindle or basaloid features. The stroma is characteristically hypocellular, exhibiting a myxoid to hyalinized appearance, occasionally resembling the pattern seen in fibroadenoma of the breast. In the current series, one case (a myoepithelial-rich PA) completely lacked the typical CAA-like areas. Additionally, a high-grade carcinoma with basaloid features exhibited fibrotic stroma and a trabecular arrangement reminiscent of CAA but demonstrated high grade cytomorphology.

Notably, areas typical of PA are only present in approximately half of the cases (11/21, 52%). When present, the proportion of typical PA is generally small (ranging from 5% to 15% in most cases) but can occasionally account for the entire tumor (as observed in case #2 of the current series). Since the typical PA area may be focal in these tumors, it is suggested to sample the tumor extensively or entirely for an accurate diagnosis. Although no PA area has been detected in the high-grade carcinoma with basaloid features, given the presence of *HMGA2::WIF1* fusion, the tumor likely represents a high-grade carcinoma (not otherwise specified) ex PA.

Immunophenotypically, all reported cases, including those from the current series, exhibit diffuse uniform immunopositivity for S100 and CK7, particularly in the CAA-like areas. The immunoexpression of myoepithelial markers varies: while Agaimy et al. reported no positivity for SMA in all 12 tumors (0%), we detected calponin positivity in focal areas or rare cells in 83% (5/6) of cases, including the CAA-like areas, suggesting that tumor cells exhibited a (modified) myoepithelial phenotype. All tumors consistently test negative for

p40 (0/18), whereas p63 is positive in 64% (9/14), predominantly in a focal and/or weak pattern.

Molecularly, the fusion occurs between *HMGA2* exon 3, 4, or 5, and *WIF1* exon 3, 4, 8, 9, or 10 [3–7]. As *HMGA2* translocation has only been reported in PA, CA ex PA, myoepithelioma (case #5), and myoepithelial carcinoma *de novo* among all salivary gland neoplasms [1, 11, 12], the detection of *HMGA2::WIF1* fusion implies a diagnosis of PA, CA ex PA, myoepithelioma, or myoepithelial carcinoma. In this study, we exclusively utilized RNA sequencing; however, other ancillary tools such as fluorescence in situ hybridization (FISH) and immunohistochemistry for HMGA2 may be considered as screening tools for this tumor.

Given the presence of prominent trabeculae and canalicular arrangement of monotonous tumor cells, the absence of typical PA areas in some cases, and the S100+/CK7+ immunoprofile, the differential diagnoses, especially in small biopsy material, may include striated duct adenoma, canalicular adenoma (if originating from minor salivary glands), basal cell adenoma, and polymorphous adenocarcinoma. Their histologic features, immunoprofile, and molecular alterations are summarized in Table 3. For instance, striated duct adenoma is a novel benign neoplasm first included in the WHO 5$^{th}$ edition of head and neck tumors [13]. It most commonly affects the parotid gland, although minor salivary gland may also be the origin of this tumor. Histologically, the tumor is composed of closely packed ducts/cysts lined by columnar cells with eosinophilic cytoplasm and exhibits a positive immunoprofile for S100/CK7 [14]. Recently, *IDH2* R172X mutations have been proposed as the defining molecular alteration for SA [15]. Due to overlapping histology, cytologic features, and the S100+/CK7 positive immunoprofile, distinguishing between salivary gland neoplasms harboring *HMGA2::WIF1* fusion with CAA-like morphology and striated duct adenoma, either histologically or immunophenotypically, can be challenging. The presence of a conventional PA component is the most useful histologic feature for distinguishing *HMGA2::WIF1* fusion-driven tumors from their diagnostic mimickers, but this component is only present in approximately 50% of cases. In challenging cases, especially those without a conventional PA component, the detection of *HMGA2::WIF1* fusion is essential for establishing a correct diagnosis.

Our series and the literature review emphasize the possibility of a malignant diagnosis and adverse outcomes in these tumors. Therefore, *HMGA2::WIF1* fusion alone does not warrant a benign diagnosis in salivary gland tumors; that determination must be made histologically.

It is noteworthy that the CAA-like pattern is not exclusive to tumors with *HMGA2::WIF1* fusion. Agaimy et al. [7] reported the CAA-like pattern in PA with *HMGA2::RPSAP52* or *HMGA2::HELB* fusion. Similarly, we detected alternative fusions in three PAs with CAA-like patterns, including *TGFBR3::PLAG1*, *HMGA2::LOC105373146*, and truncated *HMGA2* consisting of exons 1–3 only (one case each, data not shown).

In conclusion, we have presented 8 cases of salivary gland neoplasm with *HMGA2::WIF1* fusion, three of which were diagnosed as malignant. We also conducted a comprehensive literature review. Together, it is noteworthy that approximately 20% of salivary gland neoplasms with *HMGA2::WIF1* fusions are malignant and may lead to adverse events, such

as recurrence, distant metastasis and disease-related death. These tumors are characterized by cellular areas of monotonous tumor cells arranged as interconnected trabeculae/canaliculi with a hypocellular hyalinized to myxoid stroma. The detection of *HMGA2::WIF1* fusion is helpful in distinguishing them from their diagnostic mimickers, such as canalicular adenoma, striated duct adenoma, and basal cell adenoma. However, it is important to note that *HMGA2::WIF1* fusion does not exclude the possibility of malignancy.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Funding statement:

Research reported in this publication was supported in part by the Cancer Center Support Grant of the National Institutes of Health/National Cancer Institute under award number P30CA008748.

## Data Availability statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References

1. Katabi N, Ghossein R, Ho A et al. Consistent plag1 and hmga2 abnormalities distinguish carcinoma ex-pleomorphic adenoma from its de novo counterparts. Hum Pathol 2015;46;26–33. [PubMed: 25439740]
2. Stenman G Fusion oncogenes in salivary gland tumors: Molecular and clinical consequences. Head Neck Pathol 2013;7 Suppl 1;S12–19. [PubMed: 23821214]
3. Queimado L, Lopes CS, Reis AM. Wif1, an inhibitor of the wnt pathway, is rearranged in salivary gland tumors. Genes Chromosomes Cancer 2007;46;215–225. [PubMed: 17171686]
4. Persson F, Andren Y, Winnes M et al. High-resolution genomic profiling of adenomas and carcinomas of the salivary glands reveals amplification, rearrangement, and fusion of hmga2. Genes Chromosomes Cancer 2009;48;69–82. [PubMed: 18828159]
5. Ihrler S, Stiefel D, Jurmeister P et al. Salivary carcinosarcoma: Insight into multistep pathogenesis indicates uniform origin as sarcomatoid variant of carcinoma ex pleomorphic adenoma with frequent heterologous elements. Histopathology 2023;82;576–586. [PubMed: 36376255]
6. Matsuyama A, Hisaoka M, Nagao Y, Hashimoto H. Aberrant plag1 expression in pleomorphic adenomas of the salivary gland: A molecular genetic and immunohistochemical study. Virchows Arch 2011;458;583–592. [PubMed: 21394649]
7. Agaimy A, Ihrler S, Baněčková M et al. Hmga2-wif1 rearrangements characterize a distinctive subset of salivary pleomorphic adenomas with prominent trabecular (canalicular adenoma-like) morphology. Am J Surg Pathol 2022;46;190–199. [PubMed: 34324456]
8. Zhu G, Benayed R, Ho C et al. Diagnosis of known sarcoma fusions and novel fusion partners by targeted rna sequencing with identification of a recurrent actb-fosb fusion in pseudomyogenic hemangioendothelioma. Mod Pathol 2019;32;609–620. [PubMed: 30459475]
9. Antonescu CR, Agaram NP, Sung YS, Zhang L, Swanson D, Dickson BC. A distinct malignant epithelioid neoplasm with gli1 gene rearrangements, frequent s100 protein expression, and metastatic potential: Expanding the spectrum of pathologic entities with actb/malat1/ptch1-gli1 fusions. The American journal of surgical pathology 2018;42;553–560. [PubMed: 29309307]
10. Dickson BC, Hornick JL, Fletcher CDM et al. Dermatofibrosarcoma protuberans with a novel col6a3-pdgfd fusion gene and apparent predilection for breast. Genes Chromosomes Cancer 2018;57;437–445. [PubMed: 30014607]

11. Mito JK, Jo VY, Chiosea SI, Dal Cin P, Krane JF. Hmga2 is a specific immunohistochemical marker for pleomorphic adenoma and carcinoma ex-pleomorphic adenoma. Histopathology 2017;71;511–521. [PubMed: 28463429]

12. Dalin MG, Katabi N, Persson M et al. Multi-dimensional genomic analysis of myoepithelial carcinoma identifies prevalent oncogenic gene fusions. Nat Commun 2017;8;1197. [PubMed: 29084941]

13. Skálová A, Hyrcza MD, Leivo I. Update from the 5th edition of the world health organization classification of head and neck tumors: Salivary glands. Head Neck Pathol 2022;16;40–53. [PubMed: 35312980]

14. Weinreb I, Simpson RH, Skálová A et al. Ductal adenomas of salivary gland showing features of striated duct differentiation (‘striated duct adenoma’): A report of six cases. Histopathology 2010;57;707–715. [PubMed: 21054495]

15. Rooper LM, Agaimy A, Assaad A et al. Recurrent idh2 mutations in salivary gland striated duct adenoma define an expanded histologic spectrum distinct from canalicular adenoma. Am J Surg Pathol 2023;47;333–343. [PubMed: 36510691]

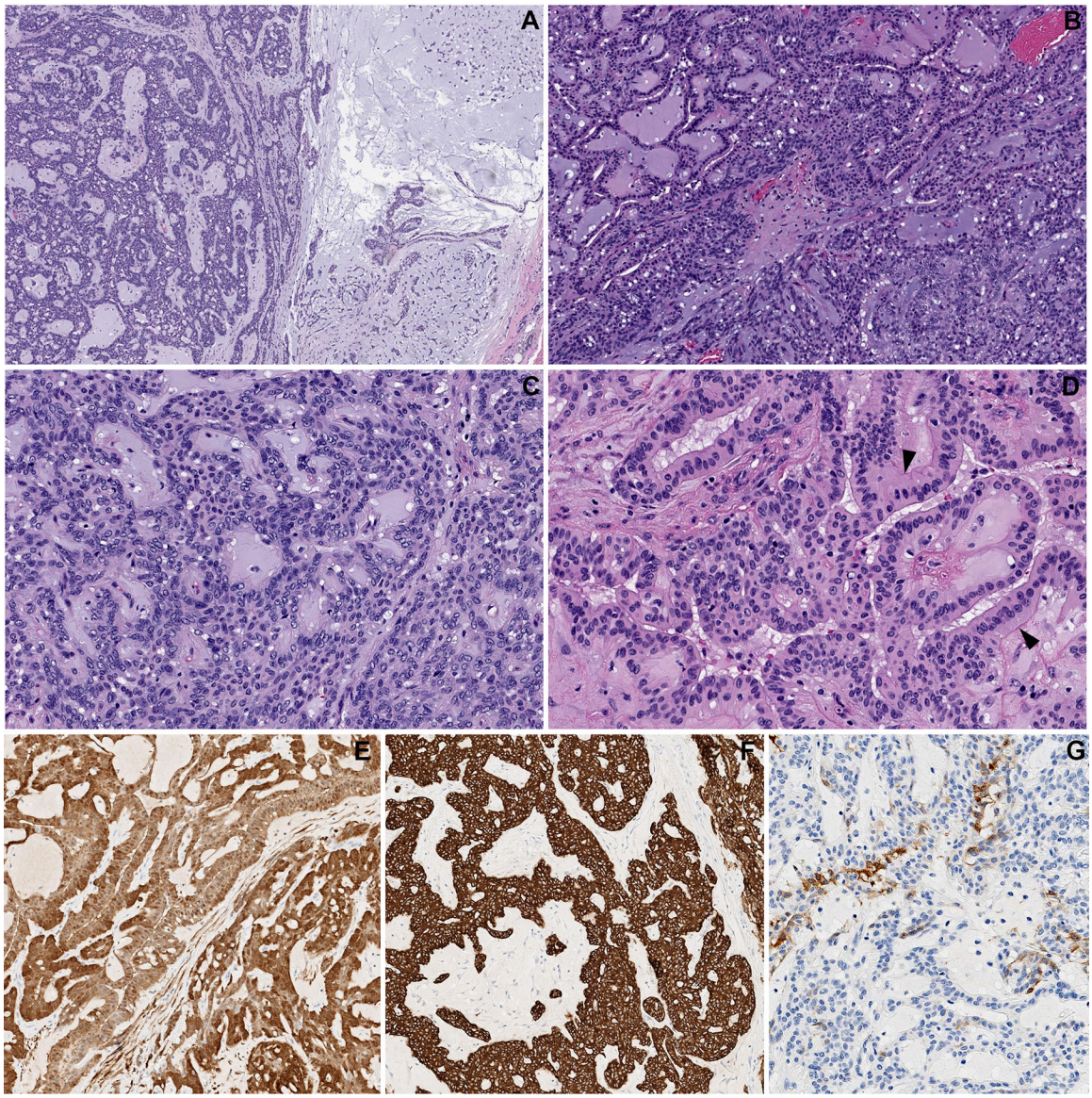



**Figure 1. A pleomorphic adenoma (PA) of the parotid gland with *HMGA2::WIF1* fusion exhibiting features reminiscent of canalicular adenoma (case #1).**

(A) At low power (40X magnification), the tumor comprises a typical area of PA (right) with abundant myxoid stroma and a cellular area (left) characterized by canalicular/trabecular architecture. (B) The cellular area consists of interconnected cords (bottom right) and canaliculi with appreciable lumens (top left). The stroma in between appears hypocellular, edematous, or myxoid (100X magnification). (C-D) The lining cells of the trabeculae and canaliculi exhibit characteristics of being cuboidal to slightly spindle with scanty cytoplasm and round to oval nuclei (C), or oncocytic with abundant eosinophilic cytoplasm, centrally located nuclei, small but conspicuous nuclei, prominent cell membrane, and a hint of cytoplasmic striation (arrowhead in D), resembling striated duct adenoma. Salivary gland tumors with *HMGA2::WIF1* fusions typically exhibit diffusely positivity for S100 (E) and CK7 (F). Calponin immunostain shows focal positivity (G).

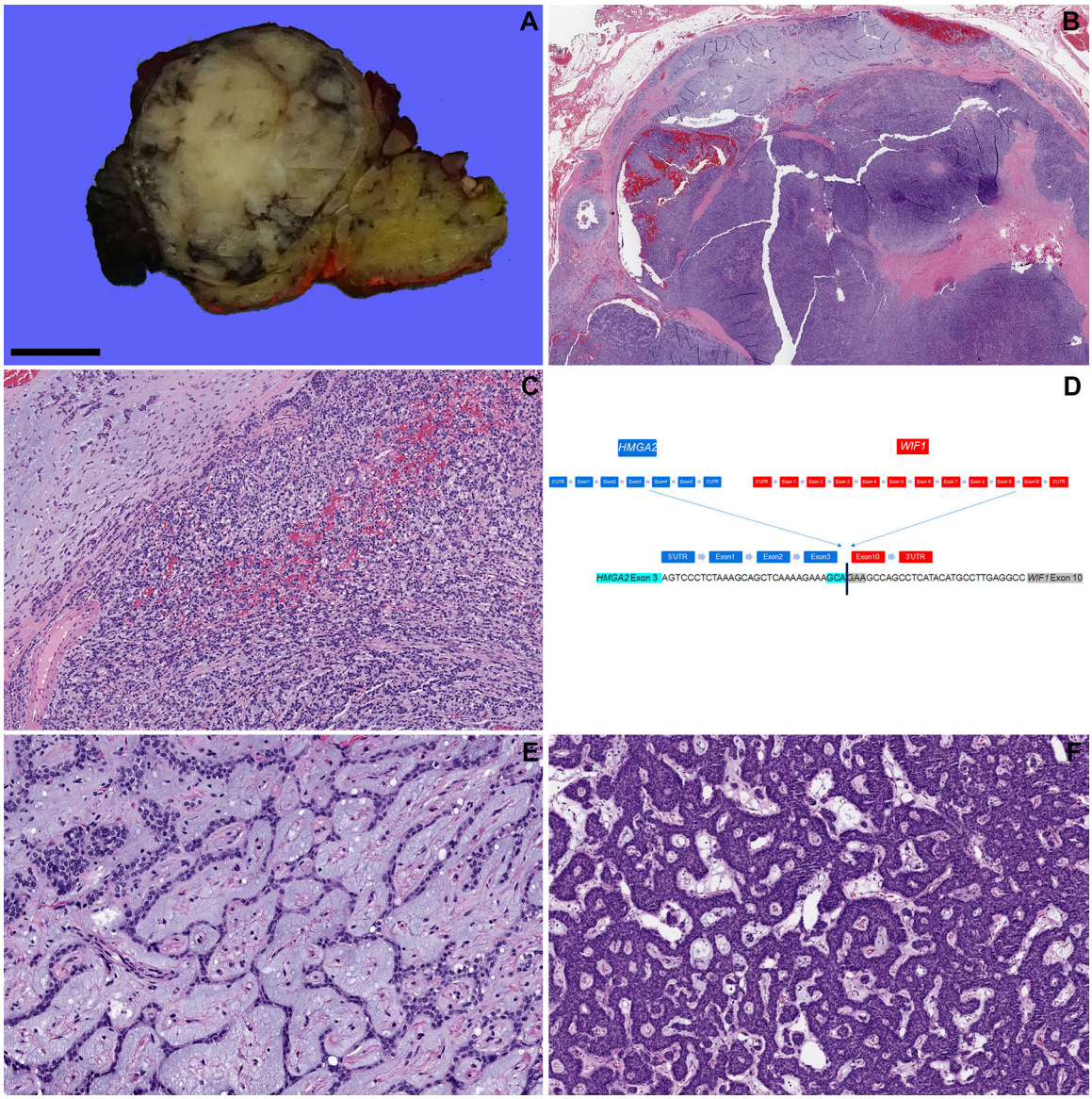



**Figure 2. Other histologic patterns observed in salivary gland neoplasms with *HMGA2::WIF1* fusion.**

(A-D) A myoepithelial-rich pleomorphic adenoma (case #2). Macroscopically, the tumor is well-circumscribed with a multinodular appearance (A). Scale bar: 1 cm. Histologically, it exhibits features typical of pleomorphic adenoma with a heterogenous arrangement of hypocellular myxoid and cellular spindle myoepithelial-rich areas (B). The cellular areas consist of sheets of spindle myoepithelial cells in a myxoid stroma. Canalicular/trabecular arrangement are not observed (C). ARCHER RNA sequencing reveals fusion between *HMGA2* exon 3 and *WIF1* exon 10 (D). (E-F) Other histologic features occasionally observed in these tumors include hyalinized or myxoid stroma lined by compressed flattened lining, resembling fibroadenoma of the breast (E), and trabeculae of basaloid cells with peripheral palisading, resembling basal cell adenoma (F).

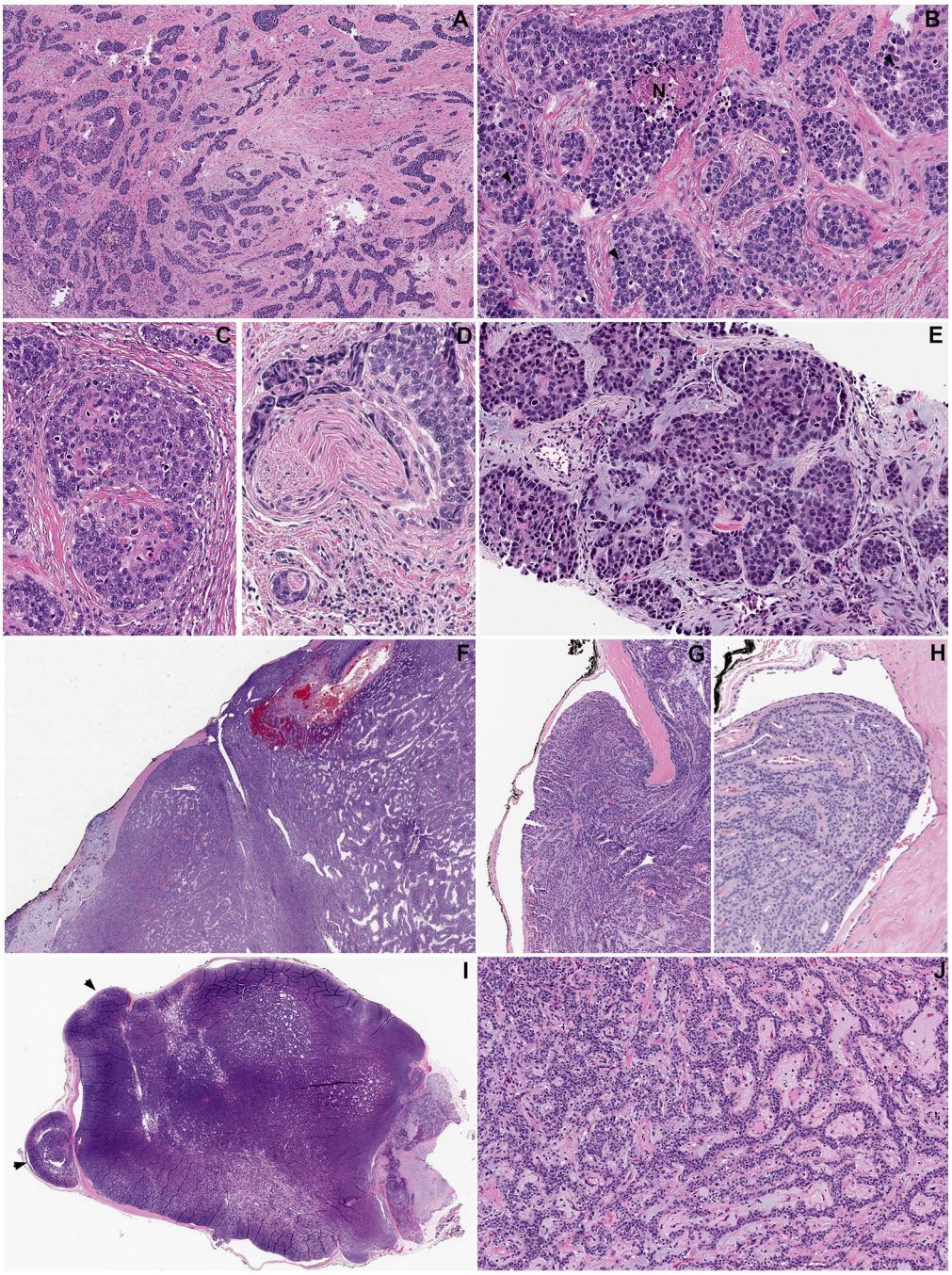



**Figure 3. Salivary gland carcinoma with *HMGA2::WIF1* fusion.**

(A-E) A high-grade carcinoma originating from the minor salivary gland of the buccal site (case #6) is composed of cords or trabeculae of tumor cells intermixed with hypocellular densely fibrotic stroma (A). At high power, the tumor exhibits basaloid features with high nuclear/cytoplasmic ratio (B). Multifocal tumor necrosis (N) is present. Focally, true squamous differentiation with intracellular keratin and keratin pearls is seen (C). The carcinoma exhibits multifocal perineural invasion (D). The distant metastasis to the lung shows a similar trabecular growth pattern and myxoid intervening stroma (E). (F-H)

A myoepithelial carcinoma ex pleomorphic adenoma (case #7). The typical pleomorphic adenoma region with abundant myxoid stroma is seen at the bottom left (F). The remaining tumor is composed of an expansile nodule of myoepithelial cells arranged as interconnected trabeculae resembling canalicular adenoma (G,H). A focus of vascular invasion (G-H) is present in which a large tumor embolus protrudes into the lumen of a large-size vessel. (I-J) A myoepithelial carcinoma ex pleomorphic adenoma (case #8). Typical areas of pleomorphic adenoma are seen on the lower right of panel I. The tumor exhibits nodular expansile growth of myoepithelial cells with numerous protrusions (arrows). At high power, the myoepithelial-rich nodules are composed of trabeculae and cords of myoepithelial cells in a hypocellular fibromyxoid stroma having a canalicular adenoma-like pattern (J).

**Table 1.**

Clinicopathologic features of salivary gland neoplasms with HMGA2::WIF1 fusion.

| N | Clinical features and outcome | | | | | Histologic characteristics | | | | | | | | Immunohistochemistry | | | | | | *HMGA2::WIF1* fusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age/ Sex | Site | Procedure | Size (cm) | Outcome | Diagnosis | Typical PA area (%) | CAA-like area | Hyalinized or edematous stroma | PNI | LVI | MI | Necrosis | S100 | Calponin | CK7 | CK5/6 | p63 | p40 | |
| 1 | 36/M | Parotid | Resection | 1.5 | NED (32m) | PA | Yes (15%) | Yes | Yes | N | N | 0 | No | + | Focal | + | NA | Focal | − | HMGA2 exon 3 WIF1 exon 9 |
| 2 | 48/M | Parotid | Resection | 3.2 | NA | PA | Yes (100%) | No | No | N | N | 0 | No | + | Rare cells | + | Focal | Rare cells | NA | HMGA2 exon 3 WIF1 exon 10 |
| 3 | 66/F | Parotid | Resection | 1.2 | NA | PA | No | Yes | Yes | N | N | 0 | No | + | NA | NA | NA | + | − | HMGA2 exon 3 WIF1 exon 10 |
| 4 | 76/F | Submandibular | Biopsy (LR) | NA | LR (NA) | PA | No | Yes | Yes | N | N | 0 | No | + | − | NA | NA | NA | − | HMGA2 exon 3 WIF1 exon 3 |
| 5 | 64/F | Parotid | Resection | 1.8 | NED (23m) | Myoepithelioma | No | Yes | Yes | N | N | 0 | No | + | focal | NA | + | NA | − | HMGA2 exon 3 WIF1 exon 9 |
| 6 | 60/F | Buccal | Resection | 1.2 | LR (5m), DM (31m), DOD (73m) | Carcinoma with basaloid features | No | Yes * | Yes | Y | N | 15 | Yes | + | NA | NA | + | Rare cells | NA | HMGA2 exon 3 WIF1 exon 3 |
| 7 | 83/F | Parapharyngeal space | Resection | 3.7 | NA | MECA ex PA | Yes (10%) | Yes | Yes | N | Y | 0 | No | + | focal | + | NA | Rare cells | − | HMGA2 exon 5 WIF1 exon 3 |
| 8 | 71/M | Parotid | Resection | 3.0 | NED (46m) | MECA ex PA | Yes (5%) | Yes | Yes | N | N | 6 | No | + | NA | NA | + | NA | − | HMGA2 exon 3 WIF1 exon 9 |

F: female, M: male, NED: no evidence of carcinoma, NA: not available, LR: local recurrence, DM: distant metastasis, m: months, PA: pleomorphic adenoma, CAA: canalicular adenoma, PNI: perineural invasion, LVI: lymphovascular invasion, N: no, Y: yes, MI: mitotic index (per 2 square mm, 10 high power fields), +: positive.

* The tumor has trabecular arrangement and hypocellular hyalinized stroma reminiscent of CAA. However, it shows high grade cytomorphology, frequent mitosis, and necrosis beyond the benign cytologic features of CAA.

**Table 2.**

Literature review: reported cases of salivary gland neoplasms with HMGA2::WIF1 fusion.

| Ref. | Age/Sex | Site | Diagnosis | *HMGA2::WIF1* fusion | PA component (%) | Outcome |
|---|---|---|---|---|---|---|
| [3] | NA | NA | PA | HMGA2 exon 3<br>WIF1 exon 4 | NA | NA |
| | | | CA ex PA | HMGA2 exon 4<br>WIF1 exon 10 | NA | NA |
| [4] | 86/F | NA | PA | HMGA2 exon 3<br>WIF1 exons 3, 4, 9 or 10<br><br>HMGA2 exon 5<br>WIF1 exon 3 | NA | NED (5y) |
| | 76/M | | PA | | | DOOC (3y) |
| | 74/F | | PA | | | NED (3y) |
| | 37/F | | PA | | | NED (3y) |
| | 73/M | | PA<br>CA ex PA (second recurrence) | | | LR (41y) (PA), LRR (46y) (CA ex PA), DOD (51y) |
| [6] | NA | NA | PA | HMGA2 exon 3<br>WIF1 exon 10 | NA | NA |
| [7] | 68/F | Parotid | PA | HMGA2 exon 3<br>WIF1 exon 10 (n=9)<br><br>HMGA2 exon 5<br>WIF1 exon 3 (n=2)<br><br>HMGA2 exon 3<br>WIF1 exon 8 (n=1) | No | NA |
| | 60/F | | | | No | NED (16m) |
| | 58/M | | | | Yes (10%) | NA |
| | 43/M | | | | No | NA |
| | 74/F | | | | No | NED (2m) |
| | 67/F | | | | Yes (10%) | NED (2m) |
| | 46/M | | | | No | NED (5m) |
| | 43/F | | | | Yes (10%) | NA |
| | 87/M | | | | Yes (5%) | NED (18m) |
| | 71/M | | | | Yes (10%) | NA |
| | 50/F | | | | Yes (50%) | NA |
| | 66/M | | | | No | NA |
| [5] | 59/M | Minor salivary glands | CA ex PA: carcinoma is adenoid cystic carcinoma with sarcomatoid transformation | *HMGA2::WIF1* (details NA) | Yes (NA) | NA |
| * | 36/M | Parotid | PA | HMGA2 exon 3<br>WIF1 exon 9 | Yes (15%) | NED (32m) |
| | 48/M | Parotid | PA | HMGA2 exon 3<br>WIF1 exon 10 | Yes (100%) | NA |

| Ref. | Age/Sex | Site | Diagnosis | *HMGA2::WIF1* fusion | PA component (%) | Outcome |
|---|---|---|---|---|---|---|
| | 66/F | Parotid | PA | HMGA2 exon 3<br>WIF1 exon 10 | No | NA |
| | 76/F | Submandibular | PA | HMGA2 exon 3<br>WIF1 exon 3 | No | LR (NA) |
| | 64/F | Parotid | Myoepithelioma | HMGA2 exon 3<br>WIF1 exon 9 | No | NED (23m) |
| | 60/F | Buccal | Carcinoma with basaloid features | HMGA2 exon 3<br>WIF1 exon 3 | No | LR (5m), DM (31m), DOD (73m) |
| | 83/F | Parapharyngeal space | MECA ex PA | HMGA2 exon 5<br>WIF1 exon 3 | Yes (10%) | NA |
| | 71/M | Parotid | MECA ex PA | HMGA2 exon 3<br>WIF1 exon 9 | Yes (5%) | NED (46m) |

*Current study, Ref.: reference, NA: not available, PA: pleomorphic adenoma, CA ex PA: carcinoma ex pleomorphic adenoma, MECA: myoepithelial carcinoma, LR: local recurrence, LRR: locoregional recurrence, DOD: dead of disease, NED: no evidence of disease, DM: distant metastasis.

**Table 3.**

Differential diagnosis of salivary gland neoplasms with HMGA2::WIF1 fusion.

| | Involved salivary glands | Tumor border | Cellular composition | Architecture | Stroma | Cytologic features | Immunohistochemistry | Molecular alterations |
|---|---|---|---|---|---|---|---|---|
| Tumor with *HMGA2::WIF1* fusion | Major > minor | Encapsulated or infiltrative | Biphasic or monophasic (one cell type) | Trabeculae or canaliculi | Myxoid, or hyalinized hypocellular | Cuboidal to columnar, pale to eosinophilic cytoplasm, cytoplasmic striation | CK7/S100 diffusely positive<br>HMGA2 positive | *HMGA2::WIF1* fusion |
| Canalicular adenoma | Minor | Encapsulated | Monophasic | Canaliculi | Minimal hypocellular | Cuboidal to columnar | CK7/S100 diffusely positive | No known alterations |
| Striated duct adenoma | Major and minor | Encapsulated | Monophasic | Compacted ducts and microcysts | Minimal hypervascular hypocellular | Columnar, eosinophilic cytoplasm, cytoplasmic striation | CK7/S100 variably positive | *IDH2* R172X mutations |
| Basal cell adenoma | Major | Encapsulated | Biphasic: composed of basaloid and luminal cells | Trabecular, tubular, solid, or membranous | Hypocellular or hypercellular myoepithelial-derived stroma | Basaloid peripheral cells<br>Cuboidal luminal cells<br>Squamoid cells | CK7/S100 diffusely positive<br>Beta-catenin nuclear | *CTNNB1* alteration<br>*CYLD1* alteration (in membranous subtype) |
| Polymorphous adenocarcinoma | Minor > major | Infiltrative | Monophasic | Single file, trabeculae, tubules, solid, cribriform, papillae | Hypocellular, myxoid, hyalinized, or fibrous | Pale nuclei, open chromatin, nuclear grooves | CK7/S100 diffusely positive | *PRKD1* hotspot mutations<br>*PRKD1*, *PRKD2*, or *PRKD3* fusion |

PA: pleomorphic adenoma.